Applicants respectfully submit that there is strong support for the claims of the present invention in the present specification. First, Applicants direct the Examiner to the Specification at page 7, lines 16-22. Second, Example 11 uses vaginal fluid, whole blood, serum, and plasma as sources for nucleases. The fact that the present invention (specifically, in the embodiment of 50% methanol/50% DMSO as an example) protected RNA in these fluids is strong evidence that nucleic acids are preserved. One skilled in the art would accept this as very convincing evidence for the claimed invention. Accordingly, withdrawal of these rejections is respectfully requested.

The Examiner has alleged that Claims 1-4,6-8,10 and 12-17 are rendered obvious under 35 U.S.C.§103 by Gee et al., Williams et al., Connelly et al., Tometsko, Evinger-Hodges et al. and Bresser et al. None of the above references, singly nor in combination teach or suggest the claimed invention.

The present invention describes a composition for stabilizing the nucleic acids of at least one cell in a specimen at ambient temperature, the composition being comprised of:

(a) a first substance capable of precipitating or denaturing proteins, comprising at least one alcohol or ketone whose concentration is less than 80% of the total composition; and

(b) a second facilitator substance to aid in the infusion of the first substance into said at lease one cell whose concentration is greater than 20% of the total composition, wherein the concentrations of said first and second

substances are effective to stabilize the nucleic acids of said at least one cell in a specimen at ambient temperature, and further, wherein the combined concentrations of said first and second substances is 100% of said composition.

The Gee et al. reference does not disclose and actually makes no mention whatsoever of stabilizing nucleic acids, which is an essential aspect of the claimed invention. Gee et al. are primarily concerned with protein structures when so-called binding pairs are considered as labels (see col. 27, lines 25-46). Gee et al. are also concerned with maintaining cellular structure and integrity (see col. 4, lines 53-56; col. 30, lines 49-51) for fluorescent staining of these structures using specific labels. In particular, Gee et al. suggest the use of permeabilization agents such as DMSO to allow bulky dye components to cross cell membranes (col. 30, lines 56-59). On the other hand, the claimed invention is not concerned with allowing penetration of the cell by large dye structures (see page 2, lines 20-40).

Most importantly, Gee et al. specifically teach that the fixation step is followed by an additional series of steps for washing, permeabilization etc. However, the claimed invention which utilizes the combination of a fixation step (with a fixation chemical) with a permeability agent (such as DMSO) is not taught or suggested by Gee et al. (see, for example col. 30, lines 38-44 and 54-60). Gee et al. suggest that additional steps, like permeabilization, can be used, but not as a single reagent combined with the "fixation" chemical(s). Thus, Applicants respectfully submit that the Examiner's statement that Gee et al.

teach a composition for stabilizing nucleic acid is clearly incorrect. Gee et al. do not teach the composition of the claimed invention, but rather, teach a method, but they certainly do not teach a method for stabilizing nucleic acids. While there may be compositions in the prior art for fixing cells, the prior art is not replete with compositions for preserving cellular nucleic acid (not fixing cells) for further analysis.

With respect to Claims 2 and 4, Gee et al. teach cold methanol, not methanol (col. 30, line 49). With respect to Claim 3, Gee et al do not use DMSO as part of a single composition with the alcohol or ketone (col. 30, line 55, "optionally followed or accompanied by").

Williams et al. teach that one may use ethanol to fix and kill M. tuberculosis and still allow the PCR reaction to take place. All this says to one skilled in the art is that ethanol at the appropriate concentrations can kill M. tuberculosis but not harm genomic M. tuberculosis DNA. It says nothing about the RNA in the same M. tuberculosis. In addition, there is no teaching for a permeation aid (like DMSO). Most importantly, Example 3 of the present invention (page 13) demonstrates that Williams et al. teach away from the claimed invention as methanol and various formulations of ethanol do not work.

Connelly et al. teach that the proper cell fixative (not preservative) needs to be alcohol free (see col. 4, line 6; col. 17, lines 14-20). Clearly the claimed invention uses alcohol! In addition, Connelly et al. are most concerned with maintaining the antigenic integrity of the cellular structures and allowing labeling

agents, such as antibodies to penetrate to the inside (see for example, Col. 4, line 64 to Col. 5, line 18). This is not a concern of the claimed invention. Finally, the main teaching of Connelly et al. is a compound for fixation of the structure R1R2R3R4R5-Ar-X, which bears no resemblance to methanol, used in the claimed invention. The Examiner is incorrect in stating that Connelly et al. teach preserving nucleic acids. This is not mentioned at all. In addition, while Connelly et al. teach that DMSO can be used as an agent that facilitates transport across cell membranes, this is only to allow large molecules to cross (much like Gee et al., discussed above) and it is in combination with the non-alcohol fixative, also taught and mentioned above.

With respect to Tometsko, the Examiner points out that Tometsko teaches a composition for stabilizing the nucleic acids of RBCs that involves methanol. However, the Examiner makes no mention of the fact that the methanol treatment in Tometsko is taught to be carried out at below 0°C (in fact, at -70°C, see col. 7, lines 5-7), and the "composition" is absolute methanol, which is not what is claimed in the claimed invention or revealed in the specification. In addition, in Tometsko, 10% DMSO is used prior to the methanol step and is clearly a separate step (see col. 6, line 66 - col. 7, line 4) and poured off prior to the addition of fixative. Thus, DMSO is not present at a concentration close to what the claimed invention requires. Nowhere is a combined alcohol or ketone with DMSO used in a single reagent and/or step as it is in the claimed invention. A final critical distinction is that Tometsko teaches its fixation procedure not

directly on the primary biological specimen, but only after the specimen has been processed. Thus, what is taught in Tometsko is not what is claimed in the claimed invention.

The reference Evinger-Hodges et al. teaches that the sample containing cells of interest must be deposited on a solid surface such as a glass microscope slide, or a strip of tape, prior to fixation (see page 6, lines 19-24; page 12, lines 12-17). This clearly is not claimed (or done) in the claimed invention. Secondly, the fixatives used in this reference vary depending on the cell type to be studied (see Examples 3,4,6 and 10 for alcohol-acetic acid, and Example 7 for methonol-acetone). There is no teaching in this reference of a preservative involving an alcohol or ketone and a facilitator as part of one composition.

The Examiner has alleged that the subject matter of Claim 6 is taught by Gee et al. in light of Tometsko. Both teach that the use of alcohol as a fixative occurs at low temperatures, not ambient temperature. Since the purpose of Gee et al. (fluorescent labels for staining cells) is not the same as the purpose of Tometsko (*in situ* hybridization involving fixed cells on a surface), a case of obviousness cannot be made, as one skilled in the art would not selectively pull an element from Gee et al. and combine it with another element from Tometsko and then use that combination at a temperature higher than that which is taught by Gee et al. or Tometsko.

Although Example 127 in Gee et al does mention that mRNA is the subject of the exercise, it does not teach how to fix and prepare mouse fibroblasts for

the exercise. It is by "standard methods," as stated in Gee et al. What are these methods? Where is one taught how to perform the fixation and preparation of mRNA? The methods taught in Gee et al., itself, as already discussed, must be assumed to be part of the "standard methods" and do not involve the composition claimed by the presently claimed invention.

RNA is the subject of several claims in Evinger-Hodges (Claims 13,14 and 15). However, the fixation and/or preparation are as given in Example 8 of this reference, and as was pointed out by the Examiner, the stabilization is by a fixative composition of different make-up and procedure than that claimed in the claimed invention (page 6, lines 24-33), and also applied to cells deposited onto a solid surface, which is not done in the claimed invention.

Bresser et al. teach rather low concentrations of the active components (2-20% DMSO, 2-20% alcohol, etc. see col. 2, lines 50-65). As was previously pointed out to the Examiner, in previous Amendments, Bresser et al. clearly teaches away from the claimed invention; and provides no additional teachings in combination with the other cited references.

It is well established that obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the combination, Carella v Starlight Archery, 804 F.2d 135, 231 U.S.P.Q. 644 (Fed. Cir. 1986). In determining obviousness, the inquiry is not whether each element existed in the prior art, but whether the prior art made obvious the invention as a whole for which

patentability is claimed. Hartness International, Inc. v Simplimatic Engineering Co., 189 F.2d 1100, 2 U.S.P.Q. 2d 1826 (Fed. Cir. 1987). Furthermore, the Examiner cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to depreciate the claimed invention, In re Fine, 837 F.2d 1071, 5 U.S.P.Q. 2d 1596 (Fed. Cir. 1988), which the Examiner has clearly done in order to reject the claims under 35 U.S.C.§103.

Applicants have carefully studied the cited art as applied by the Examiner to reject the present claims and respectfully assert that the cited art does not render the teachings of the present invention obvious to one of ordinary skill in the art. Therefore, it is believed that the rejections of the claims under 35 U.S.C.§103 is improper, and withdrawal of these rejections is respectfully requested.

Attached hereto is a marked-up version of the changes made to the claim by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claim 1 has been amended as follows.

1. (3x Amended) A composition for stabilizing the ~~[structure and]~~ nucleic acids of at least one cell in a specimen at ambient temperature, said composition being comprised of:

(a) a first substance capable of precipitating or denaturing proteins, comprising at least one alcohol or ketone whole concentration is less than 80% of the total composition; and

(b) a second facilitator substance to said in the infusion of the first substance into said at least one cell whose concentration is greater than 20% of the total composition,

wherein the concentrations of said first and second substances are effective to stabilize the ~~[structure and]~~ nucleic acids of said at least one cell in a specimen at ambient temperature, and further wherein the combined concentration of said first and second substances is 100% of said composition.